Claims

We claim:

1. A method of quantitation of glycated protein in a sample which comprises:

(a) contacting a solid support matrix which comprises a negatively charged group and a hydroxyboryl compound and which has a measurement area, with an aliquot of biological sample sufficient to cover said measurement area;

(b) contacting said solid support matrix with an aliquot of a first buffer sufficient to rinse off unbound protein wherein said first buffer has a pH selected to allow both glycated and non-glycated protein to be bound to said solid support matrix;

(c) quantitating protein bound to said measurement area using measurement of a selected property of said protein to give a first bound protein reading;

(d) contacting said solid support matrix with an aliquot of a second buffer sufficient to rinse off unbound protein wherein said second buffer has a pH selected to allow glycated protein to be bound to said solid support matrix but where non-glycated protein is not substantially bound to said solid support matrix;

(e) quantitating protein bound to said measurement area using measurement of the property measured in step (c) to give a second bound protein reading; and

(f) calculating percentage of glycated protein using said first and second bound protein readings.

2. A method according to claim 1 wherein the property measured is an optical reading.

3. A method according to claim 2 wherein the optical reading is absorbance or reflectance at a specified wavelength.

4. A method according to claim 3 wherein the glycated protein is glycated hemoglobin.

5. A method according to claim 3 wherein the glycated protein is glycated albumin.

6. A method for quantitation of amount of glycated protein in a biological sample which compromises:

(a) contacting a solid support matrix which comprises a negatively charged group and a hydroxyboryl compound and which has a measurement area with an aliquot of a biological sample sufficient to cover said measurement area;

(b) contacting said solid support matrix with an aliquot of a first buffer sufficient to rinse off unbound protein, wherein said first buffer has a pH of about 5.0 to about 7.0;

(c) quantitating protein bound to said measurement area to give a first bound protein reading;

(d) contacting said solid support matrix with an aliquot of second buffer sufficient to rinse off unbound protein, wherein said buffer has a pH of about 8.0 to about 10.0;

(e) quantitating protein bound to said measurement area to give a second bound protein reading; and

(f) calculating percentage of glycated protein in said sample using said first bound protein reading and said second bound protein reading.

7. A method according to claim 6 wherein said first and second bound protein readings measure the same property.

8. A method according to claim 7 wherein the property measured is an optical reading.

9. A method according to claim 8 wherein the optical reading is absorbance or reflectance at a specified wavelength.

10. A method according to claim 9 wherein the glycated protein is glycated hemoglobin.

11. A method according to claim 9 wherein the glycated protein is glycated albumium.

12. A method for quantitation of glycated hemoglobin in a biological sample which comprises;

(a) adding said sample to a sample application site which is in communication with a solid support matrix which comprises a negatively charged group and a dihydroxyboryl compound and which has a measurement area;

(b) adding an aliquot of a first buffer at said sample application site, wherein said first buffer has a pH between about 5.0 and about 7.0;

(c) making a first optical reading of said measurement area at a wavelength at which hemoglobin absorbs light;

(d) adding an aliquot of a second buffer at said sample application site, wherein said second buffer has a pH between about 8.0 and about 10.0;

(e) making a second optical reading of said measurement area at a wavelength at which hemoglobin absorbs light; and

(f) calculating the percentage of glycated hemoglobin in said blood sample using said first and second optical readings.

13. A method according to claim 12 wherein said dihydroxyboryl compound has the formula

$$R - B(OH)_2$$

wherein R is selected from the group consisting of phenyl, substituted hydrogen, and alkyl of 1 to about 6 carbon atoms.

14. A method according to claim 13 wherein R is selected from the group consisting of phenyl, m-aminophenyl, hydrogen, ethyl, 1-propyl and 2-methyl-1-butyl.

15. A method according to claim 14 wherein R is m-aminophenyl.

16. A method according to claim 13 wherein the negatively charged group is selected from the group consisting of carboxylate, sulfate, sulfonate, sulfinate and phosphate.

17. A method according to claim 16 wherein the negatively charged group is carboxylate.

18. A method according to claim 12 wherein said solid support matrix is selected from the group consisting of cellulose, nitrocellulose, cellulose acetate, polyacrylamide, agarose polyacrylaminde copolymer, agarose, starch, nylon, nylon polyesters, dextran, cross-linked dextran, dextran acrylamide copolymer, cross-linked hydroxyethylmethacrylate substituted cross-linked polystyrenes, and polyvinylalcohol.

19. A method according to claim 18 wherein said solid support matrix is carboxy cellulose.

20. A method according to claim 12 wherein said first buffer is selected from the group consisting of MES, MOPS and HEPES.

21. A method according to claim 12 wherein said second buffer is ammonium acetate or taurine.

22. A method for quantitation of non-hemoglobin glycated protein in a biological sample which comprises:

(a) adding said sample to a sample application site which is in communication with a solid support matrix which comprises a negatively charged group and a dihydroxyboryl compound and which has a measurement area;

(b) adding an aliquot of a first buffer to said sample application site, wherein said first buffer has a pH between about 5.0 and about 7.0;

(c) making a first optical reading of said measurement area at a wavelength at which said labeling agent absorbs light;

(d) adding an aliquot of a second buffer to said sample application site wherein said second buffer has a pH between about 8.0 and about 10.0;

(e) making a second optical reading of said measurement area at a wavelength at which said labeling agent absorbs light; and

(f) calculating the percentage of glycated protein in said sample using said first and second optical readings.

23. A method according to claim 22 wherein said dihydroxyboryl compound has the formula:

wherein R is selected from the group consisting of phenyl, substituted phenyl, hydrogen, and alkyl of 1 to about 6 carbon atoms.

24. A method according to claim 23 wherein R is selected from the group consisting of phenyl, m-amino phenyl, hydrogen, ethyl, 1-propyl and 2-methyl-1-butyl.

25. A method according to claim 23 wherein R is m-aminophenyl.

26. A method according to claim 21 wherein the negatively charged group is selected from the group

consisting of carboxylate, sulfate, sulfonate, sulfinate and phosphate.

27. A method according to claim 26 wherein the negatively charged group is carboxylate.

28. A method according to claim 22 wherein said solid support matrix is selected from the group consisting of cellulose, nitrocellulose, cellulose acetate, polyacrylamide, agarose polyacrylaminde copolymer, agarose, starch, nylon, nylon polyesters, dextran, cross-linked dextran, dextran acrylamide copolymer, cross-linked hydroxyethylmethacrylate substituted cross-linked polystyrenes, and polyvinylalcohol.

29. A method according to claim 28 wherein said solid support matrix is carboxy cellulose.

30. A method according to claim 22 wherein said first buffer is selected from the group consisting of MES, MOPS and HEPES.

31. A method according to claim 22 wherein said second buffer is ammonium acetate or taurine.

32. A method according to claim 22 wherein said sample is labeled with a protein specific labeling agent.

33. A method according to claim 22 wherein said sample is serum or plasma.

34. A method according to claim 33 wherein said non-hemoglobin glycated protein is glycated albumin.

35. A diagnostic device for quantitation of glycated hemoglobin utilizing the method according to claim 1.

36. A diagnostic device for quantitation of glycated protein utilizing the method according to claim 6.

37. A diagnostic device for quantitation of glycated protein utilizing the method according to claim 12.

38. A diagnostic device for quantitation of glycated protein utilizing the method according to claim 22.

39. A diagnostic device according to claim 22 wherein said glycated non-hemoglobin protein is albumin.

40. A kit comprising said diagnostic device according to any of claims 34 to claim 39, a first buffer having a pH of about 5.0 to about 7.0 and a second buffer having a pH of about 8.0 to about 10.0.